REFERENCES

1. Brandt CM, Sistrunk WW, Duffy MC *et al*. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. *Clin Infect Dis* 1997; **24**: 914–919.
2. Wilson MG, Kelley K, Thornhill TS. Infection as a complication of total knee-replacement arthroplasty. Risk factors and treatment in sixty-seven cases. *J Bone Joint Surg* 1990; **72**: 878–883.
3. Schoifet SD, Morrey BF. Treatment of infection after total knee arthroplasty by debridement with retention of the components. *J Bone Joint Surg Am* 1990; **72**: 1383–1390.
4. Drancourt M, Stein A, Argenson JN, Zannier A, Curvale G, Raoult D. Oral rifampin plus ofloxacin for treatment of *Staphylococcus*-infected orthopedic implants. *Antimicrob Agents Chemother* 1993; **37**: 1214–1218.
5. Javaloyas DM, Monreal PM. Oral antibiotic therapy in the adult bacterial osteomyelitis: results after two years of follow-up. *Med Clin (Barc)* 1999; **113**: 488–489.
6. Widmer AF, Gaechter A, Ochsner PE, Zimmerli W. Antimicrobial treatment of orthopedic implant-related infections with rifampin combinations. *Clin Infect Dis* 1992; **14**: 1251–1253.
7. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. *JAMA* 1998; **279**: 1537–1541.
8. Lewin CS, Amyes SG. The bactericidal activity of DR-3355, an optically active isomer of ofloxacin. *J Med Microbiol* 1989; **30**: 227–231.
9. Morris AB, Kanyok TP, Scott J *et al*. Rifamycins. In: Yu VL, Merigan TC, Barriere SL, eds, *Antimicrobial therapy and vaccines*. Philadelphia, PA: Lippencott, Williams & Wilkins, 1998; 901–962.
10. Stein A, Bataille JF, Drancourt M *et al*. Ambulatory treatment of multidrug-resistant *Staphylococcus*-infected orthopedic implants with high-dose oral co-trimoxazole (trimethoprim-sulfamethoxazole). *Antimicrob Agents Chemother* 1998; **42**: 3086–3091.
11. Bassetti M, Di Biagio A, Cenderello G *et al*. Linezolid treatment of prosthetic hip infections due to methicillin-resistant *Staphylococcus aureus* (MRSA). *J Infect* 2001; **43**: 148–149.
12. Rayner CR, Baddour LM, Birmingham MC, Norden C, Meagher AK, Schentag JJ. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. *Infection* 2004; **32**: 8–14.
13. Bassetti M, Vitale F, Melica G *et al*. Linezolid in the treatment of Gram-positive prosthetic joint infections. *J Antimicrob Chemother* 2005; **55**: 387–390.
14. Deirmengian C, Greenbaum J, Lotke PA, Booth RE, Lonner JH. Limited success with open debridement and retention of components in the treatment of acute *Staphylococcus aureus* infections after total knee arthroplasty. *J Arthroplasty* 2003; **18** (suppl 1): 22–26.

RESEARCH NOTE

Clinical significance of isolated *Staphylococcus aureus* central venous catheter tip cultures

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ABSTRACT

This retrospective cohort study examined the clinical significance of isolated *Staphylococcus aureus* central venous catheter (CVC) tip cultures (i.e., positive tip cultures without concomitant positive blood cultures). Subsequent *S. aureus* bacteraemia was found in nine (12%) of 77 patients at a median time of 4 days after CVC removal. A high co-morbidity score and no effective antibiotic treatment within 48 h of CVC removal were independent risk-factors for septic complications following multivariate analysis. A matched case-control study that compared the above cohort with patients with CVC tip cultures negative for *S. aureus* supported the significance of these findings.

Keywords Bacteraemia, central venous catheter, risk-factors, significance, *Staphylococcus aureus*, tip cultures

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Few data exist concerning the clinical significance of central venous catheter (CVC) tip cultures that are positive for *Staphylococus aureus* in patients who have no blood cultures collected around the time of CVC removal, or whose

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concomitant blood cultures do not grow *S. aureus* (i.e., isolated CVC tip cultures) [1]. The present report describes a retrospective cohort study of all adult patients with isolated *S. aureus* CVC tip cultures at two tertiary care medical centres between January 1999 and December 2004.

Cases were defined as patients who: (i) had a distal CVC tip culture that grew ≥15 *S. aureus* colonies by the roll-plate technique, and (ii) either did not have blood cultures taken, or had negative blood cultures taken between 2 days before and 1 day after CVC removal (concomitant blood cultures) [2]. A subsequent septic complication was defined as isolation of *S. aureus* with an antibiotic susceptibility pattern identical to the CVC tip isolate from a normally sterile site between 3 days and 12 weeks after CVC removal. Additionally, a matched, nested, 1:1 case-control study was performed to compare the *S. aureus* complication rate in the cases with that in a similar patient cohort with CVC tip cultures negative for *S. aureus*. Control patients were selected randomly and matched by age, Charlson index, and stay in a surgical or medical intensive care unit. The Charlson index reflects the sum of a patient's weighted co-morbidities and predicts mortality [3–5]. Therapy was considered appropriate if treatment with at least one antibiotic to which susceptibility was documented was started within 24 h before and 48 h after CVC removal (adapted from [6]). *S. aureus* processing was performed according to CLSI guidelines [7,8]. The study was approved by the institutional review boards of the hospitals concerned.

Septic complication was the outcome of interest. Bivariate analyses were conducted by Pearson's chi-square test, Fisher's exact test or the Mann–Whitney *U*-test, as appropriate, to identify factors associated with the outcome ($p < 0.05$). Variables with $p < 0.2$ were entered into a backward stepwise non-conditional logistic regression analysis to identify independent risk-factors. In the case-control study, McNemar's test and the Wilcoxon signed-rank test were used to compare categorical and continuous variables, respectively; the log-rank test was used to compare the rates of septic complications.

Of 101 isolated *S. aureus* CVC tip cultures, 24 were excluded for the following reasons: follow-up of ≤ 12 weeks or incomplete medical records ($n = 11$); invasive *S. aureus* infection in ≤12 weeks ($n = 3$); death within 48 h of CVC removal ($n = 3$); CVC tip with multiple organisms ($n = 3$); concurrent non-bacteraemic *S. aureus* infection ($n = 2$); and *S. aureus* bacteraemia 24–48 h after CVC removal ($n = 2$). The final cohort comprised 77 consecutive patients with a mean age of 59 years; nine (12%) of 77 patients developed a subsequent *S. aureus*-related complication, all of which were bacteraemia. One patient died from *S. aureus*-related septic shock.

Table 1 shows the results of the bivariate analyses. A high Charlson index, chronic obstructive pulmonary disease (COPD), and no effective antibiotic therapy received within 48 h of CVC removal, were all factors associated with septic complications. Only two (3%) of 59 patients who had received appropriate antibiotics within 48 h of CVC removal developed subsequent bacteraemia, compared with seven (39%) of 18 patients who had not received appropriate antibiotics. The median duration of antibiotic treatment in patients with no complication was 12 days.

Table 1. Risk-factors for *Staphylococcus aureus*-related septic complications among 77 patients with isolated *S. aureus* central venous catheter tip cultures

Characteristic	Cases with complications[a] ($n = 9$)	Cases without complications[a] ($n = 68$)	OR (95% CI)	p
Mean age ± SD (years)	55 ± 24	59 ± 17		> 0.2
Male gender	7 (78)	44 (65)	1.91 (0.37–9.92)	> 0.2
Stay in ICU	3 (33)	26 (38)	0.81 (0.19–3.51)	> 0.2
Co-morbid conditions				
Charlson score ≥ 5	5 (56)	10 (15)	7.25 (1.66–31.71)	0.01
Diabetes mellitus	5 (56)	17 (25)	3.75 (0.90–15.59)	0.11
COPD	5 (56)	10 (15)	7.25 (1.66–31.71)	0.01
Haemodialysis dependency	0 (0)	3 (4)	–	> 0.2
Current malignancy	3 (33)	20 (29)	1.20 (0.27–5.28)	> 0.2
Intravascular device[b]	2 (22)	12 (18)	1.33 (0.25–7.23)	> 0.2
Orthopaedic device	0 (0)	10 (15)	–	> 0.2
Previous surgery[c]	4 (44)	34 (50)	0.80 (0.20–3.24)	> 0.2
CVC exit site infection	3 (33)	28 (41)	0.71 (0.17–3.10)	> 0.2
SIRS[d]	4 (44)	39 (57)	0.59 (0.15–2.41)	> 0.2
Non-tunnelled CVC[e]	7 (78)	54 (79)	0.91 (0.17–4.86)	> 0.2
Concomitant blood cultures performed	4 (44)	40 (59)	0.56 (0.14–2.27)	> 0.2
MRSA	6 (67)	41 (60)	1.32 (0.30–5.72)	> 0.2
No effective antibiotics by day 2	7 (78)	11 (16)	18.14 (3.32–99.16)	< 0.001
28-day mortality rate	1 (11)	6 (9)	1.29 (0.14–12.15)	> 0.2

ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; SIRS, systemic inflammatory response syndrome; MRSA, methicillin-resistant *Staphylococcus aureus*.

[a]Total number (percentage) or mean ± SD.

[b]Inferior vena cava filter ($n = 5$), pacemaker or implantable cardiac defibrillator ($n = 3$), synthetic intravascular graft ($n = 2$), prosthetic heart valve ($n = 2$), coronary artery stent ($n = 1$), transjugular intrahepatic portosystemic shunt ($n = 1$).

[c]Within the previous 6 months.

[d]See [17].

[e]Other: tunnelled CVCs ($n = 7$), totally implantable devices ($n = 7$), and peripherally inserted central catheters ($n = 2$).

Table 2. Comparison of patient characteristics in the matched nested case-control study

Characteristic	Cases (%) (n = 77)	Controls (%) (n = 77)	p
Mean age ± SD (years)[a]	59 ± 18	59 ± 15	> 0.2
Male gender	51 (66)	47 (61)	> 0.2
Stay in ICU[a]	29 (38)	29 (38)	> 0.2
Co-morbid conditions			
Median Charlson score (range)[a]	2 (0–11)	2 (0–10)	> 0.2
Diabetes mellitus	22 (29)	16 (21)	> 0.2
COPD	15 (20)	19 (25)	> 0.2
Chronic liver disease	8 (10)	8 (10)	> 0.2
Haemodialysis dependence	3 (4)	2 (3)	> 0.2
Current malignancy	23 (30)	29 (38)	> 0.2
Intravascular device[b]	14 (18)	12 (16)	> 0.2
Orthopaedic device	10 (13)	3 (4)	0.15
CVC exit site infection	31 (40)	17 (22)	0.06
SIRS	43 (56)	40 (52)	> 0.2
Non-tunnelled CVC	61 (79)	63 (82)	> 0.2
Days of hospitalisation after CVC tip culture obtained (range)	9 (0–82)	14 (0–122)	> 0.2
Complicating *Staphylococcus aureus* bacteraemia[c]	9 (12)	2 (2)	0.03
28-day mortality rate	7 (9)	11 (14)	> 0.2

ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; SIRS, systemic inflammatory response syndrome.
[a]These variables were used in the matching process.
[b]Inferior vena cava filter (n = 8), pacemaker or implantable cardiac defibrillator (n = 7), synthetic intravascular graft (n = 5), prosthetic heart valve (n = 3), intravascular stent (n = 2), transjugular intrahepatic portosystemic shunt (n = 1).
[c]≤ 12 weeks.

Logistic regression analysis revealed two independent risk-factors for septic complications, namely a Charlson index ≥5 (adjusted OR 9.07; 95% CI 1.42–57.92; p 0.02) and no effective antibiotic therapy received within 48 h of CVC removal (adjusted OR 21.23; 95% CI 3.20–140.77; p 0.002).

Table 2 shows the results of the case-control study. Only two (2%) control patients, compared with nine (12%) case patients, developed septic complications (p 0.03); these occurred at a median of 62 and 4 days, respectively, after CVC removal (p 0.03).

S. aureus catheter tip cultures are accompanied frequently by positive blood cultures [9,10], and findings at the institutions in the present study, in which 267 (73%) of 368 *S. aureus* CVC tip cultures were associated with concurrent bacteraemia, agree with this observation. However, the clinical significance of isolated *S. aureus* CVC tip cultures has not been defined [1]. To our knowledge, the present study is the first to investigate specifically the outcome of patients with this clinical entity. Nine (12%) patients with isolated *S. aureus* CVC tip cultures were identified who developed bacteraemia between 3 and 33 days after catheter removal. A high Charlson index and a failure to receive effective antibiotics within 48 h of CVC removal were the only independent risk-factors for subsequent septic complications. The latter also remained the only independent predictor in an analysis of a subgroup of 44 patients who had negative concomitant blood cultures (data not shown). This finding, together with the results of the matched case-control study, supports the conclusion that the subsequent bacteraemic episodes signify a genuine complication, rather than merely non-detection of *S. aureus* bacteraemia at the time of CVC removal or the existence of an independent healthcare-related complication.

COPD has not been associated with *S. aureus* bacteraemia in previous reports; its significance in bivariate analysis may be an artefactual finding caused by multiple testing. The majority (57%) of non-tunnelled CVCs was inserted into the subclavian vein. Catheter location and rate of septic complications were not related (p > 0.2). The pathogenic mechanisms responsible for subsequent *S. aureus* septic complications are unknown. The potential role of small, clinically non-evident, intravascular thrombi, or low-grade bacteraemia caused by mechanical manipulation at the time of CVC removal, remain to be determined [11].

There were limitations to the study. Blood and CVC tip isolates were not available for molecular typing [12,13], so their relatedness could not be confirmed. Only 21% of the patients underwent echocardiography; therefore, endocarditis may have been under-diagnosed [14,15]. The study was also limited by its retrospective design; however, strict inclusion criteria were used to minimise sampling, misclassification and surveillance bias [16].

In summary, isolated *S. aureus* CVC tip cultures were associated with a significant risk of septic complications. If validated, the data would indicate that patients with this clinical entity should receive a course of anti-staphylococcal antibiotics, the optimal duration of which remains to be determined.

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REFERENCES

1. Mermel LA, Farr BM, Sherertz RJ, Raad II, O'Grady N, Harris JS. Guidelines for the management of intravascular catheter-related infections. *Clin Infect Dis* 2001; **32**: 1249–1272.
2. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous catheter-related infection. *N Engl J Med* 1977; **296**: 1305–1309.
3. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**: 373–383.
4. Lesens O, Methlin C, Hansmann Y *et al.* Role of comorbidity in mortality related to *Staphylococcus aureus* bacteremia: a prospective study using the Charlson weighted index of comorbidity. *Infect Control Hosp Epidemiol* 2003; **24**: 890–896.
5. Beddhu S, Bruns FJ, Saul M *et al.* A simple comorbidity scale predicts clinical outcomes and costs in dialysis patients. *Am J Med* 2000; **108**: 609–613.
6. Fowler VG, Olsen MK, Corey R *et al.* Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Int Med* 2003; **163**: 2066–2072.
7. NCCLS. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 6th edn. Approved standard M7-A6. Wayne, PA: NCCLS, 2003.
8. NCCLS. *Performance standards for antimicrobial susceptibility testing*. 13th informational supplement M100–S13. Wayne, PA: NCCLS, 2003.
9. Cercenado E, Ena J, Rodriguez-Creixems M, Romero I, Bouza E. A conservative procedure for the diagnosis of catheter-related infections. *Arch Intern Med* 1990; **150**: 1417–1420.
10. Peacock SJ, Eddleston M, Emptage A, King A, Crook DW. Positive intravenous line tip cultures as predictors of bacteraemia. *J Hosp Infect* 1998; **40**: 35–38.
11. Raad I, Narro J, Khan A, Tarrand J, Vartivarian S, Bodey GP. Serious complications of vascular catheter-related *Staphylococcus aureus* bacteremia in cancer patients. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 675–682.
12. Tenover FC, Arbeit RD, Goering RV *et al.* 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
13. Khatib R, Sharma M, Naqvi SA *et al.* Molecular analysis of *Staphylococcus aureus* blood isolates shows lack of polyclonal bacteremia. *J Clin Microbiol* 2003; **41**: 1717–1719.
14. Fätkenheuer G, Cornely O, Seifert H. Clinical management of catheter-related infections. *Clin Microbiol Infect* 2002; **8**: 545–550.
15. Fowler VG, Li J, Corey GR *et al.* Role of echocardiography in evaluation of patients with *Staphylococcus aureus* bacteremia: experience in 103 patients. *J Am Coll Cardiol* 1997; **30**: 1072–1078.
16. Jernigan JA, Farr BM. Short-course therapy of catheter-related *Staphylococcus aureus* bacteremia: a meta-analysis. *Ann Intern Med* 1993; **119**: 304–311.
17. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; **20**: 864–874.

RESEARCH NOTE

Evaluation of differential gene expression in susceptible and resistant clinical isolates of *Klebsiella pneumoniae* by DNA microarray analysis

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ABSTRACT

DNA microarray technology was used to evaluate differential gene expression in a susceptible *Klebsiella pneumoniae* isolate and a resistant clinical derivative. Nineteen genes were up-regulated in the resistant isolate when compared with the susceptible isolate. An ABC transporter-related gene, *ycjV*, was strongly over-expressed, suggesting the existence of a novel active efflux mechanism. Approximately half of the up-regulated genes coded for ribosomal proteins, or proteins involved in tRNA metabolism. Among 33 down-regulated genes, almost one-third were related to nitrogen metabolism. A possible role of fitness in the development of antimicrobial resistance is suggested.

Keywords Antimicrobial resistance, efflux pumps, gene regulation, *Klebsiella pneumoniae*, microarray, transcriptional analysis

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